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# *Staphylococcus saprophyticus* β-Lactamase Production and Disk Diffusion Susceptibility Testing for Three β-Lactam Antimicrobial Agents

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**β-Lactamase production and MIC determinations for penicillin, methicillin, and cephalothin were assessed for 67 strains of *Staphylococcus saprophyticus* and correlated with results of disk diffusion susceptibility testing. Fifty-five (82%) of the 67 strains produced β-lactamase, and 40 (77%) of these β-lactamase-producing strains were susceptible (zone size, >29 mm) by disk diffusion techniques. Although the range of zone sizes for β-lactamase producers was broad (26 to 36 mm), all 38 strains with a zone size of <31 mm by disk diffusion testing were β-lactamase producers compared with 17 (59%) of 29 with larger zone sizes ($P$ = 0.0000008). The median penicillin MIC for 12 *S. saprophyticus* strains was 0.25 μg/ml and was not related to β-lactamase production. Although the methicillin MICs for 15 strains were in the susceptible range (4.0 μg/ml), interpretation of disk diffusion testing for oxacillin varied greatly among laboratories using identically prepared media and standardized techniques. Criteria presently used to define susceptibility of *Staphylococcus aureus* to penicillin and oxacillin by disk diffusion are inappropriate for *S. saprophyticus*. The clinical significance of the β-lactamase produced by these strains needs further evaluation.**

*Staphylococcus saprophyticus*, a coagulase-negative staphylococcus, is a common cause of acute urinary infections in young women (3, 6, 15). Unlike most other coagulase-negative staphylococci, *S. saprophyticus* strains are reported to be uniformly susceptible to most antibiotics active against gram-positive organisms (3, 9, 15). Yet, in a recent study of urinary infections with this organism among college women (6), we found that the majority of strains were not susceptible to penicillin and methicillin by disk diffusion (DD) techniques, but were susceptible to cephalothin. To explain this observation, we assessed the prevalence of β-lactamase production among strains of *S. saprophyticus*, determined the MICs of penicillin, methicillin, and cephalothin for these strains, and correlated these results with susceptibility testing by DD.

## MATERIALS AND METHODS

Sixty-seven *S. saprophyticus* strains isolated from ambulatory patients with acute urinary infections were evaluated. Cultures were performed in one of three laboratories: the Harborview Medical Center Infectious Diseases Research Laboratory; the University of Washington Clinical Microbiology Laboratory; and the Pacific Medical Center Microbiology Laboratory. All *S. saprophyticus* strains were identified with the API Staph-Ident system (Analytab Products, Plainview, N.Y.) (4).

DD antimicrobial susceptibility testing was performed by a standard technique in accordance with published guidelines (8). In addition, during DD testing of each strain for penicillin susceptibility, the margin of the inhibitory zone was noted to be either sharp, well demarcated, and slightly heaped up or to have a tapered growth pattern. For both *Staphylococcus aureus* and *Staphylococcus epidermidis*, the former characteristic has been proposed as an indicator of β-lactamase production (1). Fifteen *S. saprophyticus* strains were tested in parallel by the University of Washington and Pacific Medical Center laboratory personnel for susceptibility to oxacillin by DD with media prepared by the same person and with identical growth conditions; neither laboratory was aware of the other's results.

MICs of penicillin, methicillin, and cephalothin for *S. saprophyticus* strains were determined by the agar dilution method with a Steers replicator delivering $10^4$ CFU to the specific antibiotic-containing Mueller-Hinton medium (15). Culture plates were evaluated for inhibition of growth after 18 h of incubation at 35°C. *S. aureus* ATCC 25923 and a laboratory strain of methicillin-resistant *S. aureus* were used as controls.

*S. saprophyticus* strains were tested for β-lactamase production by the chromogenic cephalosporin method (14) and the cloverleaf method with *Micrococcus lutea* ATCC 9341 as the indicator strain (11). For both tests, organisms were grown overnight on Mueller-Hinton agar supplemented with 0.6 μg of methicillin per ml. For the chromogenic cephalosporin test, reactions were performed in microdilution plates with 0.05 ml of cephalosporin substrate (nitrocefin) and a loop of organisms taken directly from the agar plate added to each sample cup. Microdilution plates were then covered with Parafilm (American Can Co., Marathon Products, Neenah, Wis.) and kept at room temperature. *S. aureus* ATCC 25923 and a known β-lactamase-producing *S. aureus* were used as negative and positive controls, respectively. Interpretation of test results was made after 4 h.

For the cloverleaf assay, plates containing Mueller-Hinton agar were inoculated with the indicator strain as if performing Kirby-Bauer DD testing (8) and were allowed to air dry. Plates were than streaked with one *S. saprophyticus* strain in a + pattern and a 10-μg penicillin G disk was placed at the crossing point. Cultures were incubated overnight at 35°C.

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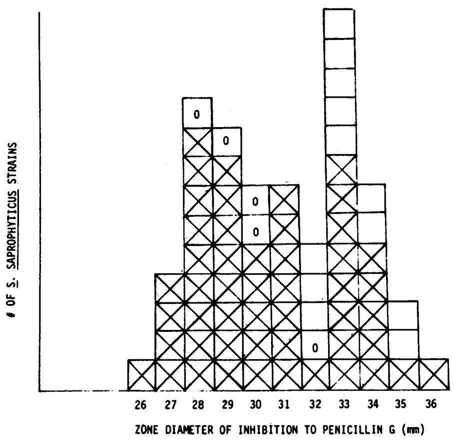


FIG. 1. Zone diameter of inhibition to penicillin G by DD for β-lactamase-positive and -negative *S. saprophyticus* strains. Symbols: ☒, β-lactamase positive, chromogenic cephalosporin positive; ▣, β-lactamase positive, chromogenic cephalosporin negative, cloverleaf positive; □, β-lactamase negative.

Control strains of *S. aureus* were those used in the chromogenic cephalosporin assay described above. All tests were performed without knowledge of the results of other tests.

Statistical testing was performed by Fisher's exact test.

## RESULTS

Fifty (75%) of the 67 *S. saprophyticus* strains produced β-lactamase as determined by the chromogenic cephalosporin method; 44 (88%) of these 50 were also positive by the cloverleaf method. An additional five strains were positive by the cloverleaf method, but failed to react with the chromogenic cephalosporin reagent. No evidence for β-lactamase production was observed for the remaining 12 (18%) *S. saprophyticus* strains.

Zone diameters of inhibition observed for the 67 strains of *S. saprophyticus* with DD testing for penicillin G ranged from 26 to 36 mm (Fig. 1). By the DD criterion presently used to define susceptibility of staphylococcus to penicillin, 52 (78%) of these strains were susceptible (zone size, >29 mm) and the remainder were in the intermediate range. Thirty-six (69%) of these 52 strains were β-lactamase producers as determined by chromogenic cephalosporin assay; 4 additional strains were positive by the cloverleaf assay only. Despite the absence of a clear breakpoint that distinguishes β-lactamase-producing strains from those that are penicillin susceptible, β-lactamase-producing strains had smaller zone sizes with DD. All 38 strains with zone sizes by DD of 31 mm or less were β-lactamase producers compared with only 17 (59%) of 29 with greater zones of inhibition ($P = 0.0000008$).

Thirty-eight (69%) of the 55 β-lactamase producing strains were noted to have sharp zone margins on DD testing with penicillin compared with 0 of the 12 non β-lactamase producers ($P = 0.0000008$). All five strains that were positive by the cloverleaf test but did not react with the chromogenic cephalosporin produced a sharp zone margin. Only two strains with zone size larger than 31 mm had a sharp edge and produced β-lactamase.

Fifteen *S. saprophyticus* strains, all with methicillin MICs of 4.0 μg/ml, were tested for oxacillin susceptibility by DD in both the University of Washington and Pacific Medical Center laboratories. The average difference in zone diameters of inhibition noted by the two laboratories was 2.7 mm (range, 0 to 4 mm). Despite these small differences, interpretation of DD testing for the 15 strains varied greatly between laboratories; 14 of the 15 strains were judged sensitive (zone size, >13 mm) by University of Washington laboratory personnel, and 1 was intermediate (zone size, 11 to 12 mm), whereas Pacific Medical Center personnel reported only 3 sensitive, 5 intermediate, and 7 resistant strains (zone size, <10 mm).

MICs of penicillin were determined for 12 *S. saprophyticus* strains, 7 β-lactamase positive and 5 β-lactamase negative. One β-lactamase-producing strain each had a penicillin MIC of 0.125 and 0.5 μg/ml, respectively; the 10 remaining strains had penicillin MICs of 0.25 μg/ml. Sixty-two (93%) of 67 strains tested had methicillin MICs of 4.0 μg/ml, 2 had methicillin MICs of 8.0 μg/ml, and 3 had methicillin MICs of 2.0 μg/ml. Sixty-one (91%) of 67 strains tested had cephalothin MICs of 0.5 μg/ml, 2 had cephalothin MICs of 0.25 μg/ml, and 4 had cephalothin MICs of 1.0 μg/ml.

## DISCUSSION

β-Lactamase production is common among staphylococci and is the major mechanism of penicillin resistance by these organisms, yet previous investigators (2, 3, 15) have failed to detect β-lactamase among strains of *S. saprophyticus*, implying susceptibility to penicillins. In contrast, we observed β-lactamase production by 55 (82%) of 67 *S. saprophyticus* strains tested. These discrepant observations may be attributable to different methods used for the detection of β-lactamase. As in our study, earlier investigators (2, 15) found the majority of strains to be positive with the cloverleaf assay, which is extremely sensitive for detection of β-lactamase, but will also be positive if penicillin is inactivated by mechanisms other than hydrolysis of the β-lactam ring. Because more specific tests for β-lactamase production were negative in these earlier reports, the results of the cloverleaf assay were attributed to production of substances other than β-lactamase by these strains (2). Similarily, the majority of our strains (49 of 67, 73%) gave positive results with the cloverleaf assay, but in contrast to previous studies (2, 15), 75% of these strains also reacted with the chromogenic cephalosporin, a specific indicator of β-lactamase production. Unlike previous studies (2, 15), we induced the production of β-lactamase by growing *S. saprophyticus* strains in the presence of subinhibitory concentrations of methicillin. Hovelius and Mårdh (2) grew the organisms in the presence of benzylpenicillin and were unable to detect production of this enzyme by strains of *S. saprophyticus*, but the inactivation of penicillin by β-lactamase removes the stimulus for enzyme production, making it a less efficient inducer of β-lactamase production than methicillin (10). Using methods similar to ours, Richardson and Marples (13) recently reported β-lactamase production in 65 (81%) of 80 strains collected since the late 1960s, supporting our findings and further suggesting that the ability of this organism to produce β-lactamase is not a newly acquired phenomenon. Regional differences in the ability of these organisms to produce β-lactamase may also occur and would explain the discrepant observations noted in this and previous studies (2, 3, 5, 13, 15). These potential differences need to be assessed to better define the epidemiology of β-lactamase production by *S. saprophyticus*. Moreover, investigations into the genetic mechanisms of β-lactamase production by this organism and the substrate profile of this enzyme are essential to determine its clinical significance and are presently being pursued.

Although the majority of strains we tested produced β-lactamase, no strains were judged resistant to penicillin G by DD testing, suggesting that the criterion presently used to define susceptibility for this coagulase-negative staphylococci may be misleading. Unfortunately, β-lactamase-producing strains produced zone sizes of 26 to 36 mm with no clear breakpoint that would differentiate β-lactamase-positive and -negative strains. However, *S. saprophyticus* strains producing zone sizes by DD of 31 mm or less are highly associated with β-lactamase production. Similarly, the appearance of a sharp edge correlated with β-lactamase production, but was seen primarily with strains producing zone sizes of 31 mm or less. Since 40 (73%) of the 55 β-lactamase-producing strains were noted to have a sharp edge or zone size of 31 mm or less with DD testing, whereas no non-β-lactamase producing strains had these characteristics, it seems prudent to report these strains as resistant to penicillin.

As with penicillin, problems with methicillin susceptibility testing of *S. saprophyticus* relate to the presently used DD criterion. In this study, 7 of 15 strains tested by DD in one laboratory were judged resistant to oxacillin despite having MICs that confirmed their susceptibility to this class of antibiotic. In contrast, when these same 15 strains were tested in another laboratory with identically prepared media and standardized techniques, no resistance was noted by DD. Differences in zone sizes observed by each laboratory for individual strains was less than 3 mm, which is acceptable interlaboratory variation for interpreting DD tests. However, with the presently used DD criteria to determine susceptibility of *S. saprophyticus* to oxacillin, this amount of variation produced faulty information. Recently recommended changes in disk potencies and interpretive zone diameter criteria may improve discrimination between methicillin-resistant and -susceptible staphylococci with DD testing, but need further evaluation (7).

In our laboratory and others, coagulase-positive and -negative staphylococci that are resistant to oxacillin are also reported as resistant to cephalothin (8). Because of this practice and the difficulty of accurately interpreting results of DD testing for oxacillin, we initially reported a number of our strains as resistant to this cephalosporin, yet subsequent MIC determinations have confirmed the susceptibility of these strains to cephalothin.

The MICs of penicillin G for *S. saprophyticus* strains in this report are similar to those noted by previous investigators (9, 12) and indicate intermediate susceptibility to this agent (14). Moreover, the MIC was independent of the ability of strains to produce β-lactamase. Although MICs of ampicillin, a commonly used agent for treatment of urinary infections, were not determined in this study, strains with similar penicillin MICs have been reported to have ampicillin MICs of <0.125 to 1 μg/ml (9). Normal doses of ampicillin readily surpass these levels in the urinary tract and probably provide adequate therapy for these infections. At present, no clinical evidence exists that suggests that treatment of these infections with β-lactamase-sensitive antimicrobial agents leads to treatment failures, but adequate studies addressing the subject are lacking. Since *S. saprophyticus* organisms have a propensity to infect the upper urinary tract (6), alternative therapies may be advisable in these patients until the clinical significance of β-lactamase production by these strains is further evaluated.

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## LITERATURE CITED

1. **Gill, V. J., L. B. Manning, and C. M. Ingalls.** 1981. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcus beta-lactamase production. J. Clin. Microbiol. **14**:437–440.
2. **Hovelius, B., and P. Mårdh.** 1977. On the diagnosis of coagulase-negative staphylococci with emphasis on *Staphylococcus saprophyticus*. Acta Pathol. Microbiol. Scand. **85**:427–434.
3. **Jordan, P. A., A. Iravani, G. A. Richard, and H. Baer.** 1980. Urinary tract infection caused by *Staphylococcus saprophyticus*. J. Infect. Dis. **142**:510–515.
4. **Kloos, W. E., and J. F. Wolfshohl.** 1982. Identification of *Staphylococcus* species with the API staph-ident system. J. Clin. Microbiol. **16**:509–516.
5. **Larsson, P. G., and K. Melby.** 1981. Antibiotic susceptibility pattern of community strains of the genus staphylococcus with special reference to *S. saprophyticus*. Natl. Inst. Public Health Ann. **4**:61–68.
6. **Latham, R. H., K. Running, and W. E. Stamm.** 1983. Urinary tract infections due to *Staphylococcus saprophyticus*. J. Am. Med. Assoc. **250**:3063–3066.
7. **McDougal, L. K., and C. Thornsberry.** 1984. New recommendations for disk diffusion antimicrobial susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. J. Clin. Microbiol. **19**:482–488.
8. **National Committee for Clinical Laboratory Standards.** 1979. Performance standards for antimicrobial disc susceptibility tests. Approved standard: ASM-2, revised. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Nicolle, L. E., and G. R. M. Harding.** 1982. Susceptibility of clinical isolates of *Staphylococcus saprophyticus* to fifteen commonly used antimicrobial agents. Antimicrob. Agents Chemother. **22**:895–896.
10. **Novick, R. P.** 1962. Staphylococcal penicillinase and the new penicillins. Biochem. J. **83**:229–235.
11. **Orstavik, I., and K. Odegaard.** 1971. A simple test for penicillinase production in *Staphylococcus aureus*. Acta Pathol. Microbiol. Scand. **79**:855–856.
12. **Price, S. G., and D. J. Flourroy.** 1982. Comparison of antimicrobial susceptibility patterns among coagulase-negative staphylococci. Antimicrob. Agents Chemother. **21**:436–440.
13. **Richardson, J. F., and R. R. Marples.** 1980. Differences in antibiotic susceptibility between *Staphylococcus epidermis* and *Staphylococcus saprophyticus*. J. Antimicrob. Chemother. **6**:499–510.
14. **Thornsberry, C., T. L. Gavan, and E. H. Gerlach.** 1977. New developments in antimicrobial agent susceptibility testing, p. 1–3. *In* J. C. Sherris (ed.) Cumulative techniques and procedures in clinical microbiology. American Society for Microbiology, Washington, D.C.
15. **Wallmark, G., I. Arremark, and B. Telander.** 1978. *Staphylococcus saprophyticus*: a frequent cause of urinary tract infection among female outpatients. J. Infect. Dis. **138**:791–797.
16. **Washington, J. A., and V. L. Sutter.** 1980. Dilution susceptibility test: Agar and macro-broth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. J. Hausler, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.